PATENT COOPERATION TREATY

PCT

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
RM/X89676/PC-EBR		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IT00/00373	21/09/2000	21/09/1999
International Patent Classification (IPC) or national classification and IPC C12N5/00		
Applicant UNIVERSITA DEGLI STUDI DI ROMA "LA SAPIENZA" et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 6 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
18/04/2001	20.11.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Fayos, C Telephone No. +49 89 2399 2180

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IT00/00373

I. Basis f the r port

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-77 as originally filed

Claims, No.:

1-27 with telefax of 07/11/2001

Drawings, sheets:

1/1 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-27
	No:	Claims	-
Inventive step (IS)	Yes:	Claims	1-27
	No:	Claims	-
Industrial applicability (IA)	Yes:	Claims	1-27
	No:	Claims	-

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

R It m V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1- Reference is made to the following documents:

D1: RICHMAN C M ET AL: 'INTERFERON PROTECTS NORMAL HUMAN GRANULOCYTE-MACROPHAGE COLONY-FORMING CELLS FROM ARA-C CYTOTOXICITY' JOURNAL OF BIOLOGICAL RESPONSE MODIFIERS, vol. 9, no. 6, 1990, pages 570-575, XP002165025 ISSN: 0732-6580

D2: EP-A-0 297 946 (UNIV SYRACUSE) 4 January 1989 (1989-01-04)

D3: SASSE FLORENZ ET AL: 'The chondramides: Cytostatic agents from myxobacteria acting on the actin cytoskeleton.' JOURNAL OF THE NATIONAL CANCER INSTITUTE (BETHESDA), vol. 90, no. 20, 21 October 1998 (1998-10-21), pages 1559-1563, XP000990945 ISSN: 0027-8874

D4: WO 97 10242 A (PHARMA MAR SA ;RUFFLES GRAHAM KEITH (GB); HIGA TATSUO (JP); TANAKA) 20 March 1997 (1997-03-20)

NOVELTY - Art. 33 (1) and (2) PCT

2- Claims 1-27 appear to be novel over the prior art cited in the search report.

2.1- The novel feature appears to be the combination of a protective compound and a chemotherapeutic compound as defined in e. g. claim 1 of the present application.

INVENTIVE STEP - Art. 33 (1) and (3) PCT

3- Claims 1-27 appear to be inventive over the prior art cited in the search report.

3.1- The closest prior art is represented by D1. D1 teaches the capability of IFN of protecting CFU-GM from Ara-C cytotoxicity.

The document reports that 1h incubation of normal CFU-GM with alpha, beta or gamma IFN followed by 3h exposure to Ara-C increased the survival of normal cells. However, increasing the Ara-C and IFN exposure time to 24h resulted in decreased

CFU-GM survival and neither alpha nor beta IFN ameliorated the toxicity. Exposure to IFN alone for these more prolonged periods was toxic, in contrast to the lack of IFN toxicity seen with 4h of total exposure time.

The closest prior art differs from the present application in that it does not disclose the combination of chemotherapeutic compound and protective compound of claim 1 of the present application.

Furthermore, malignant cells having inactive p53 pathway are not mentioned in D1 (this feature represents a criterion for discriminating between the pathological conditions in which protection is to be expected from those in which no protection will occur).

In addition, D1 reports that 1h incubation with IFN increases the percent survival of normal cells upon treatment with Ara-C, which does not mean that such cells maintain their physiological ability to proliferate.

Finally, D1 reports that incubation with IFN for more than 1h increases the cytotoxicity of IFN and abolishes the protective effect on normal cells treated with Ara-C. As the protection occurs when the cell cycle of normal cells is blocked in the G0/G1 phase and as the normal cells in physiological cells are not synchronized, a short incubation would protect only a limited number of cells. On the reverse, the pretreatment with the protective compounds of the present application may run for as many hours as a complete cell-cycle or even more without affecting the protection, thus enabling the protection of a much higher percentage of normal cells.

The technical effect achieved in the present application is an improved protection of proliferating normal cells from the eradicative action of a chemotherapeutic compound.

The objective problem posed in the present application is to provide improved means for protecting normal proliferating cells from the eradicative action of a chemotherapeutic compound.

The solution proposed is the method of e. g. claim 1.

3.2- D1 suggests that the protective effect of IFN is related to its capability of blocking cell cycle in G0/G1 phase. However, since an incubation longer than 1h with IFN results in the loss of the protective effect, either IFN does not stably block the cell-cycle in G0/G1, or if this is the case, the block in G0/G1 phase is not the reason for the protection caused by IFN on normal cells, since if this were true, the longer the incubation, the higher would the number of cells in G0/G1 phase, the better the protection.

Hence, the skilled man would not have concluded from the technical data of D1 any cause/ effect relationship between the block in G0/G1 phase and the protection of normal cells and hence would not have combined the teachings of D1 with those of any of D2, D3 or D4.

The concept underlying the present application appears therefore to be inventive and claims 1-27 appear to present an inventive step.

Re Item VIII

Certain observations on the international application

4- Claims 1, 12 are not clearly formulated (Art. 6 PCT).

CLAIMS

1. A method for protecting proliferating normal cells in an *in vitro* culture comprising said proliferating normal cells and tumor cells having an inactive p53 pathway, from the eradicative action of a chemotherapeutic compound having the capability of

- exerting a cytotoxic action toward actively proliferating cells and
- not affecting survival and proliferative potential of interphase cells,

comprising

a combined treatment including administering to said culture a protective compound having the capability of

- reversibly inhibiting cytodieresis of normal cells and
- non-inhibiting the biological action of said chemotherapeutic compound,

in combination with said chemotherapeutic compound, the administration of said protective compound resulting in the protection of at least part of said proliferating normal cells.

2. The method according to claim 1, wherein, before said combined treatment, a pre-treatment is carried out, comprising administering to said culture a protective compound as defined in claim 1, the administration of said protective compound resulting in the arrest at interphase of at least part of said normal cells.

3. The method according to claim 1 or 2, wherein, after the combined treatment, a post treatment is carried out, including a washing step wherein

- after interrupting the administration of said class B compound, said class B compound is washed off the culture, the administration of said class A compound being maintained.

4. The method according to any of claims 1 to 3, wherein the combined treatment is carried out for a time greater than or equal to the cell cycle duration of said

tumor cells having an inactivated p53 pathway.

5. The method according to any of claims 2 to 4, wherein the pre-treatment is carried out for a time greater than or equal to the cell cycle duration of said proliferating normal cell.

6. The method according to any of claims 3 to 5, wherein said washing step is carried out for a time greater than or equal to 3 hours.

7. The method according to any of claims 1 to 6, wherein said combined treatment, pre-treatment and/or post-treatment is repeated twice or more.

8. The method according to any of claims 1 to 7, wherein said protective compound is selected from the group consisting of cytochalasins, with the exclusion of cytochalasin B, jasplakinolides, chondramides, isoindolinones, and latrunculines.

9. The method according to claim 8, wherein said protective compound is selected from the group consisting of the cytochalasin D, dihydrocytochalasin B, jasplakinolide, chondramide B and latrunculin B.

10. The method according to any of claims 1 to 9, wherein said chemotherapeutic compound is selected from the group consisting of folate inhibitors, nucleoside analogues, nucleotide syntesis inhibitors, vinca alkaloids, taxanes, colchicine derivatives, podophillotoxin derivatives, and topoisomerase inhibitors.

11. The method according to claim 10, wherein said chemotherapeutic compound is selected from the group consisting of trifluorothymidine, cytarabine, 6-thioguanine, 6-mercaptoputrine, gemcytabine, fludarabine, floxuridine, ftorafur, methotrexate, trimetrexate, raltitrexed, edatrexate, lometrexol, hydroxyurea, vincristine, vinblastine, vinorelbin, vindesine, paclitaxel, docetaxel, irinotecan, topotecan, 9-amino-S(20)-camptothecine.

12. Use of a protective compound defined in claim 1

for the preparation of a pharmaceutical composition suitable as an adjuvant in a treatment of a tumor form having an inactivated p53 pathway.

13. Use according to claim 12, wherein said tumor form is a tumor form having a low proliferating potential.

14. Use according to claim 12, wherein said tumor form is an hyperproliferative lesion caused by papillomavirus.

15. Use of a protective compound defined in claim 1 for the preparation of pharmaceutical compositions suitable as an adjuvant in a treatment of a pathological infection caused by microorganisms displaying no p53 function.

16. Use of a protective compound defined in claim 1 for the preparation of pharmaceutical compositions suitable in a treatment of halopecia associated to a systemic treatment with a chemotherapeutic compound defined in claim 1.

17. A pharmaceutical product comprising a therapeutically effective amount of a protective compound as defined in claim 1, a therapeutically effective amount of a chemotherapeutic compound as defined in claim 1, and a pharmaceutically acceptable vehicle, carrier or auxiliary agent.

18. A pharmaceutical composition suitable as an adjuvant in a treatment of a tumor form having an inactive p53 function, comprising a therapeutically effective amount of a protective compound as defined in claim 1, and a pharmaceutically acceptable vehicle, carrier or auxiliary agent.

19. A pharmaceutical composition suitable as an adjuvant in a treatment of a tumor form according to claim 18, wherein said tumor form is a hyperproliferative lesion caused by papillomavirus.

20. A pharmaceutical composition suitable as an adjuvant in a treatment of a pathological infection

assaociated to microorganisms having no p53 function, comprising a therapeutically effective amount of a protective compound as defined in claim 1, and a pharmaceutically acceptable vehicle, carrier or auxiliary agent.

21. A pharmaceutical composition suitable in a treatment of halopecia associated to systemic therapy with a chemotherapeutic compound as defined in claim 1, comprising a therapeutically effective amount of a protective compound as defined in claim 1, and a pharmaceutically acceptable vehicle, carrier or auxiliary agent.

22. The pharmaceutical composition according to any of claims 17 to 21 wherein said protective compound is selected from the group consisting of cytochalasins, with the exclusion of cytochalasin B, jasplakinolides, chondramides, isoindolinones, and latrunculines.

23. The pharmaceutical composition according to claim 22, wherein said protective compound is selected from the group consisting of the cytochalasin D, dihydrocytochalasin B, jasplakinolide, chondramide B and latrunculin B.

24. The pharmaceutical composition according to any of claims 17 to 23, further comprising a therapeutical effective amount of a chemotherapeutic compound as defined in claim 1.

25. The pharmaceutical composition according to claim 24 wherein said chemotherapeutic compound is selected from the group consisting of folate inhibitors, nucleoside analogs, nucleotide synthesis inhibitors, vinca alkaloids, taxanes, colchicine derivatives, podophillotoxin derivatives, and topoisomerase inhibitors.

26. The pharmaceutical composition according to claim 25, wherein said chemotherapeutic compound is selected from the group consisting of trifluorothymidine, cytarabine, 6-thioguanine, 6-mercaptoputrine,

gemcytabine, fludarabine, floxuridine, ftorafur, methotrexate, trimetrexate, raltitrexed, edatrexate, lometrexol, hydroxyurea, vincristine, vinblastine, vinorelbin, vindesine, paclitaxel, docetaxel, irinotecan, topotecan, 9-amino-S(20)-camptothecine.

27. A kit of parts for selectively eradicating cells having an inactive p53 pathway and selectively protecting proliferating normal cells comprising:

- a protective compound as defined in claim 1;
- a chemotherapeutic compound as defined in claim 1;

as a combined preparation for simultaneous, separate or sequential use in the *in vivo* and/or *ex vivo* therapy of a tumor form having an inactive p53 pathway.

28. A kit of parts according to claim 27, wherein said tumor form is an hyperproliferative lesion caused by papillomavirus infection.

29. A kit of parts for selectively eradicating a microorganism having no p53 function and selectively protecting proliferating normal cells comprising:

- a protective compound as defined in claim 1;
- a chemotherapeutic compound as defined in claim 1;

as a combined preparation for simultaneous, separate or sequential use in the therapy of a pathological infection associated to a microorganism having no p53 function.

30. A kit of parts for selectively eradicating cells having an inactive p53 function and selectively protecting proliferating normal cells comprising:

- a protective compound as defined in claim 1;
- a chemotherapeutic compound as defined in claim 1;

as a combined preparation for simultaneous, separate or sequential use in the method according to any of claims 1 to 11.

31. Use of C3H10T1/2 cells, or cells derived therefrom, for deriving a method to eradicate tumor cells

having an inactive p53 pathway from coltures comprising said tumor cells having an inactive p53 pathway and normal cells.

32. Use according to claim 31, wherein said method is a method for a treatment of a tumor form having an inactive p53 pathway.

33. Use of C3H10T1/2 cells, or cells derived therefrom, for identifying a protective compound and/or a chemotherapeutic compound as defined in claim 1.